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(21) International Application Number: PCT/US95/07224 (22) International Filing Date: 7 June 1995 (07.06.95) (30) Priority Data: PD94A000139 26 July 1994 (26.07.94) IT (71) Applicant (for all designated States except US): FIDIA ADVANCED BIOPOLYMERS, S.R.L. [IT/IT]; Via Ponte della Fabbrica, 3/A, I-35031 Abano Terme (IT). (72) Inventors; and (75) Inventors/Applicants (for US only): PARK, Kinam [US/US]; 455 Lagrange Street, West Lafayette, IN 47905 (US). PAPARELLA, Annamaria [IT/IT]; Via Principo Amedeo, 320, I-70100 Bari (IT). BENEDETTI, Luca [IT/IT]; Via Durando, 26, I-36100 Vicenza (IT). (74) Agents: MEIKLE, Andrew, D. et al.; Birch, Stewart, Kolasch & Birch, P.O. Box 747, Falls Church, VA 22040-0747 (US).		(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG). Published <i>With international search report.</i>	
(54) Title: SYNTHESIS OF CHEMICAL GELS FROM POLYELECTROLYTE POLYSACCHARIDES BY GAMMA-IRRADIATION (57) Abstract <p>A method for the synthesis for chemical gels or hydrogels from polyelectrolyte polysaccharides includes the steps of functionalizing the polyelectrolyte polysaccharide in order to introduce double bonds into the structure thereof; and subjecting the functionalized polysaccharide to gamma-irradiation in order to produce the hydrogel which constitutes a crosslinked structure. The produced hydrogel may be formed to be biocompatible and may be used as a slow release system for drugs.</p>			

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OBJECT OF THE INVENTION

The present invention is directed to a method for the synthesis of chemical gels or hydrogels by gamma-irradiation, starting with polyelectrolyte polysaccharides, such as alginic acid, hyaluronic acid and the esters thereof. The present invention is also directed to the chemical gel or hydrogel produced by this method. Gels synthesized by the described method can be used as slow release systems for drugs.

Gels for pharmaceutical use are generally prepared from polymers by chemical means, such as by cross-linking the polymeric chain.

A method has previously been described in the literature, whereby it is possible to obtain chemical gels by gamma-irradiation, starting from water-soluble polymers such as dextran and gelatin ("Preparation and Characterization of Enzyme-Digestible Hydrogels from Natural Polymers by Gamma-irradiation", K. Kamath and K.

Park, ACS Symposium Series, 545, 55-65, 1994). According to the procedure described in the Kamath et al article, when polymers are functionalized with glycidyl acrylate to introduce double bonds into their polymeric structure, and exposed to gamma-irradiation, they give rise to chemical gels.

It has not yet been possible, however, to apply this method to polyelectrolyte polysaccharides. Indeed, it is well known that exposure to gamma-irradiation can lead to degradation of the polymeric chain with consequent loss of viscosity ("The radiation-induced degradation of hyaluronic acid", D. Deeble, G. O'Phillips, E. Bothe, H. P. Schuchmann and C. von Sonntag, Radiat. Phys. Chem., vol. 37, N. 1, 115-118, 1991).

SUMMARY OF THE INVENTION

Accordingly, it is an object of the present invention to provide a method for synthesizing chemical gels from polyelectrolyte polysaccharides using gamma-irradiation.

It is another object of the present invention to provide a chemical gel or hydrogel product which is biocompatible and which constitutes polyelectrolyte polysaccharide chains crosslinked using gamma-irradiation.

These and other objects of the present invention are fulfilled by providing a method for synthesizing

biocompatible chemical gels or hydrogels which includes reacting polyelectrolyte polysaccharides with a functionalizing agent in order to introduce double bonds into the structure so as to produce functionalized polyelectrolyte polysaccharides; and subjecting the functionalized polyelectrolyte polysaccharides to gamma-irradiation in order to produce the chemical gels.

BRIEF DESCRIPTION OF DRAWINGS

Figures 1, 2 and 3 show the conditions needed for the formation of gels from alginic acid, hyaluronic acid, and the 25% benzyl ester of hyaluronic acid.

Figure 1 is a graph showing the effect of the duration of gamma-irradiation and the effect of the concentration of sodium alginate on gel formation.

Figure 2 is a graph showing the effect of the duration of gamma-irradiation and of the concentration of hyaluronic acid on gel formation.

Figure 3 is a graph showing the affect of the duration of gamma-irradiation and of the concentration of the 25% benzyl ester of hyaluronic acid (HA-25) on gel formation.

Figure 4 is a schematic diagram which shows in the upper portion a representation of the functionalization reaction between a polysaccharide (PS) with glycidyl acrylate; and in the lower portion a representation of the formation of one type of crosslinkage between two functionalized polysaccharides that are subjected to

gamma-irradiation.

DETAILED DESCRIPTION OF THE INVENTION

The present invention has made it possible to synthesize chemical gels or hydrogels from polyelectrolyte polysaccharides using gamma-irradiation.

Polyelectrolyte polysaccharides are natural polymeric substances which contain ionic constituents. Polyelectrolyte polysaccharides tend to swell when placed in water in order to minimize the repulsion between like positive or negative charges of the ionic constituents. These properties result in the so-called "polyelectrolyte" effect. Examples of polyelectrolyte polysaccharides include alginic acid, alginates, hyaluronic acid, hyaluronic acid esters including benzyl esters, polysialic acid, gellan, xanthane, welan, pectin, and glycosaminoglycans including chondroitin sulphates, heparin sulphates, etc., as well as derivatives of these polysaccharides. These polyelectrolyte polysaccharides contrast with other natural polymeric substances, such as dextran and gelatin, which do not exhibit similar electrolytic properties and do not have the same chemical structure containing such ionic constituents.

The functionalization of polysaccharides with a functionalizing agent such as glycidyl acrylate in an aqueous medium and at ambient temperature causes double bonds to be introduced into the polymeric structure of

the polysaccharides. Solutions of the polymers, purified with an excess of glycidyl acrylate by dialysis followed by gamma-irradiation, give rise to chemical gels. Indeed, because of the presence of double bonds
5 and under the effect of the absorbed radiation, the functionalized polymers behave like macromolecules and like cross-linked products. Gels can, therefore, be formed without the addition of any outside cross-linking agents and the gels obtained do not need any further
10 purification ("Preparation and Characterization of Enzyme-Digestible Hydrogels from Natural Polymers by Gamma-Irradiation", K. Kamath and K. Park, ACS Symposium Series, 545, 55-65, 1994).

In order to accomplish the present invention,
15 important changes have been made to the method of gel synthesis by gamma-irradiation as previously described for dextran and gelatin ("Preparation and Characterization of Enzyme-Digestible Hydrogels from Natural Polymers by Gamma-irradiation", K. Kamath and K.
20 Park, ACS Symposium Series, 545, 55-65, 1994). The method of the present invention provides for acidification of solutions of polyelectrolyte polysaccharides which have been functionalized with glycidyl acrylate, within a suitable pH range.
25 Following irradiation, the acidified solutions give rise to the formation of gels at lower concentrations of polymers and for shorter irradiation times.

In accordance with the present invention, the functionalization reaction for introducing double bonds into the polyelectrolyte polysaccharides involved the use of an appropriate functionalizing agent, such as glycidyl acrylate. As schematically illustrated in Figure 4, the upper reaction illustrates the reaction between glycidyl acrylate and the hydroxy group of a polyelectrolyte polysaccharides (i.e. "PS"). The reaction product results in the introduction of a double bond into the structure of the polyelectrolyte polysaccharide. This same reaction takes place between glycidyl acrylate and the carboxy groups of the polyelectrolyte polysaccharide. Figure 4 shows in the lower reaction scheme a simplified illustration of the functionalized polyelectrolyte polysaccharide containing a number "n" of double bonds which is subjected to gamma-irradiation in order to form one type of a crosslinked structure after the opening of the double bonds and the formation of radicals. The resulting crosslinks constitute aliphatic chains of a given length and result in the formation of gels having a three-dimensional structure which exhibits certain strength and resistance properties. Since not all of the carboxy groups of the polyelectrolyte polysaccharides are involved in the formation of ester bonds with the functionalizing agent, the resulting gels may be defined as polyelectrolytes having unique properties including a significant dependence on the pH and a significant

dependence on the ionic strength of the aqueous medium in which the resulting gels are allowed to swell.

The method of synthesis of gels is conducted using solutions of polyelectrolyte polysaccharides at various concentrations. For example, the concentration range of 1-3% w/v may be used for alginic acid, hyaluronic acid and benzyl esters of hyaluronic acid with 25% esterification.

The solutions of polyelectrolyte polysaccharides may be functionalized using different quantities of an appropriate functionalizing agent such as glycidyl acrylate, for example in the range of 0.8-1.3 ml/1 g of polyelectrolyte polysaccharide.

Acidification of the polyelectrolyte polysaccharides in solution may be performed by the addition of a suitable acid, such as citric acid, hydrochloric acid or acetic acid, preferably without significantly altering the initial concentration of the polyelectrolyte polysaccharide. The polyelectrolyte polysaccharides are acidified in a pH range of from 1 to 6, more preferably a pH range of from 1 to 5, still more preferably of from 3 to 6, and most preferably between 2.5 and 3.5, before being subjected to gamma-irradiation. The acidification of the polyelectrolyte polysaccharide solution makes it possible to obtain gel formation at lower concentrations of the polymer and lower doses of irradiation.

The polyelectrolyte polysaccharides are subjected to gamma-irradiation at a preferable dose range of from at least 0.01 to 0.5 Mrad, more preferably from 0.05 to 0.5 Mrad, and most preferably from about 0.0606 to 0.485 Mrad. The time periods during which the polyelectrolyte polysaccharides are subjected to gamma-irradiation is generally in the range of from 1 to 8 hours. For example, the gamma-irradiation dose may be 0.0606 Mrad/h for time intervals varying from about 1 to 8 hours. In addition to being subjected to gamma-irradiation after acidification, the polyelectrolyte polysaccharides may also be subjected to direct gamma-irradiation before acidification. It is preferable not to increase the irradiation dose above 0.5 Mrad because of the degradation effects that may occur. For example, the swelling ratio (Q) of the gels increases at doses of gamma-irradiation at 0.485 Mrad which suggests some degradation of the crosslinked structure of the gels. Also, if the produced gels are intended to be used in "drug delivery" systems, the use of lower doses of irradiation minimizes the possibility of damaging the biological activity of the active drug or principle incorporated into the gel.

The gels thus obtained swell when placed in water. Some of the synthesized gels described below were characterized by calculating their equilibrium swelling ratio (Q), based on the weight of the dry gel and that which has swollen to equilibrium (W. S. W. Shalaby, K.

Park, Pharm. Res., 7, 816-823, 1990).

It has been found that the formation of gels depends on the concentration of the polyelectrolyte polysaccharide, on the gamma-irradiation time, and on
5 the degree of functionalization of the polyelectrolyte polysaccharide, this factor being directly linked with the quantity of glycidyl acrylate used during the functionalization reaction, and on the pH of the solution. Figures 1, 2 and 3 show the conditions needed
10 for the formation of gels, in the case of alginic acid, hyaluronic acid and the 25% benzyl ester of hyaluronic acid (HA-25), respectively. The lines in Figures 1-3 indicate the minimum concentration needed for the formation of three-dimensional gels at set gamma--
15 irradiation times. The areas above the lines represents the concentration conditions and irradiation times at which the formation of three-dimensional gels is possible. The areas below the lines represent the conditions in which the gels are not formed.

20 Regarding the symbols in the Figures, Figure 1 shows the pH of the solution at pH=3(block symbol) and pH=6(open symbol); and the quantity of glycidyl acrylate used to modify the alginate at 0.8 ml(circle) and 1.3 ml(square). In Figure 2, the pH of the solution shown
25 is pH=3(square) and pH=6(circle). In Figure 3, the pH of the solutions shown are pH=3(square) and pH=6(circle).

It has generally been found that by increasing the concentration of glycidyl acrylate and that of the polymer, it is possible to obtain the formation of gels after shorter exposure to gamma-irradiation. However, 5 the longer the gamma-irradiation time, the greater the concentration of polymer necessary for the formation of a gel. This indicates a tendency for the polymers to degrade under the effects of irradiation.

On the other hand, it has been shown that the 10 acidification of said polymer solutions leads to faster gel formation. When solutions of 1% sodium alginate w/v are acidified, they give rise to the formation of gels after only 1 hour of irradiation, while higher concentrations are necessary for solutions with pH=6 15 (2.5%). The same results were found with low-molecular-weight hyaluronic acid and its 25% benzyl ester. The polysaccharides mentioned here are polyelectrolytes characterized by the presence of carboxy groups. Acidification of solutions of these polymers cause the 20 formation of inter- and intra-chain hydrogen bonds and, consequently, the formation of stronger chemical gels following irradiation.

The acidification of such solutions before irradiation, therefore, allows the formation of gels 25 with lower polymer concentrations and shorter irradiation times, thus avoiding possible degradation of the polymer.

The gels synthesized according to the methods of the present invention can be used in the fields of medicine, health care, surgery and cosmetics, as well as in reconstructive and cosmetic surgery. For example, gels in the form of films or membranes can be used in various medical fields, such as in ophthalmology, dermatology, otorhinolaryngology and neurology, as tissue substitutes or organ coatings, as well as in tissue and organ transplants. Moreover, the gels can generally be used as a biocompatible material in cell cultures in three-dimensional systems, as well as in the form of fibers or threads for surgical suture, or in the form of gauzes for wound dressings. The gels of the present invention preferably include crosslinkage throughout the entire product such that all the polyelectrolyte molecules crosslink to form a single chemical entity.

Another important use for the compounds obtained by the method of synthesis of the present invention is as a controlled release system of one or more active principles, such as proteins, growth factors, enzymes, drugs or biologically active substances for oral, topical, s.c., i.m. or i.v. administration. Indeed, according to the dose of gamma-irradiation used, it is possible to obtain three-dimensional gels for use as drug release systems for topical or oral administration, or viscoelastic solutions in order to administer by the subcutaneous, intramuscular or intravenous routes.

The biocompatible characteristics of the chemical gels or hydrogels of the present invention allows for their employment in potential and actual biomedical applications similar to those of conventional hydrogels, such as coatings for sutures, catheters, IUD's, blood detoxicants, electrode Sensors, vascular grafts, electrophoresis cells and cell culture substrates; homogeneous-type materials including electrophoresis gels, contact lenses, artificial corneas, vitreous humor replacements, estrous-inducers, breast or other soft tissue substitutes, burn dressings, bone ingrowth sponges, dentures, ear drum plugs, synthetic cartilages, hemodialysis membranes and particulate carriers of tumor antibodies; and devices such as enzyme therapeutic systems, artificial organs and drug delivery systems.

The incorporation of the active principle in the gel can be achieved either by swelling the dry product in an aqueous solution containing the compound to be incorporated, or by mixing the active principle with the functionalized and purified polymer solution, and then by irradiating the same to obtain a gel. This last method is particularly useful when large molecules are to be incorporated, such as peptides or proteins, which would be unlikely to penetrate a gel left to swell in an aqueous solution. It is, therefore, important to synthesize gels at low doses of gamma-irradiation, so as not to alter the biological activity of the incorporated drug. The possibility of obtaining gels with low doses

of gamma-irradiation, as in the case of acidic solutions of the polyelectrolyte polysaccharides used in the present invention can, therefore, constitute an important advantage for the possible incorporation of
5 drugs. Also, the release of the drug from the gel can be controlled by the rate of degradation of the polymeric matrix.

Below are some examples of the preparation of gels from polysaccharides according to the present invention.

10 EXAMPLE 1:

1 gr of sodium alginate (medium viscosity) was dissolved in 20 ml of deionized, distilled water (5% w/v). To this solution was added 1.3 ml of glycidyl acrylate. The reaction was performed at ambient
15 temperature while stirring constantly. After 24 hours, the reaction was blocked by the addition of 6.5 ml of glycine at 20% w/v. The solution was then stirred for another 30 minutes, dialyzed for 48 hours in deionized, distilled water and lastly centrifuged at 3,000 rpm for
20 about 20 minutes. The final concentration of the solution proved to be about 1% w/v and the pH was 6. Solutions at concentrations of 1.5, 2, 2.5 and 3% were obtained by concentrating to 1% in a rotating evaporator.

25 The purified solutions of functionalized polymer were then gamma-irradiated at a dose of 0.606 Mrad/h for periods varying between 1 and 8 hours (0.0606-0.4848

Mrad). The minimum concentrations necessary for the formation of gels at the irradiation times indicated above are reported in Fig. 1.

EXAMPLE 2:

5 1 gr of sodium alginate (medium viscosity) was dissolved in 20 ml of deionized distilled water (5% w/v). To this solution was added 1.3 ml of glycidyl acrylate. The reaction was performed at ambient temperature while stirring constantly. After 24 hours,
10 the reaction was blocked by the addition of 6.5 ml of glycine at 20% w/v. The solution was then stirred for another 30 minutes, dialyzed for 48 hours in deionized distilled water and lastly centrifuged at 3,000 rpm for about 20 minutes. The final concentration of the
15 solution proved to be about 1% w/v and the pH was 6. Solutions at concentrations of 1.5, 2, 2.5 and 3% were obtained by concentrating the solution to 1% in a rotor evaporator. The solutions, at varying concentrations were acidified to pH=3 by the addition of citric acid,
20 so as to avoid any significant changes in the initial concentration of the polymer.

The purified solutions of functionalized polymer were then gamma-irradiated at a dose of 0.0606 Mrad/h for periods varying between 1 and 8 hours (0.0606-0.4848
25 Mrad). The minimum concentrations necessary for the formation of gels at the irradiation times indicated above are reported in Fig. 1.

The three-dimensional gels obtained from alginic acid were cut (1 cm^3) and dried at ambient temperature for 24 hours and at a temperature of 60°C for 12 hours. The samples, in threes, were weighed dry, left to swell in deionized, distilled water until their equilibrium had been reached, and then weighed again in their completely swollen state. The Q values relative to 1 and 8 hours of gamma-irradiation were 4.5-5.55 for 3% gels and 5.6-6.5 for 2.5% gels. The longer the gamma-irradiation time, the higher the Q value.

EXAMPLE 3:

1 gr of sodium alginate (medium viscosity) was dissolved in 20 ml of deionized, distilled water (5% w/v). To this solution was added 0.8 ml of glycidyl acrylate. The reaction was performed at ambient temperature while stirring constantly. After 24 hours, the reaction was blocked by the addition of 4 ml of glycine at 20% w/v. The solution was then stirred for another 30 minutes, dialyzed for 48 hours in deionized, distilled water and lastly centrifuged at 3,000 rpm for about 20 minutes. The final concentration of the solution proved to be about 1% w/v and the pH was 6. Solutions at concentrations of 1.5, 2, 2.5 and 3% were obtained by concentrating the solution to 1% in a rotor evaporator.

The purified solutions of functionalized polymer were then gamma-irradiated at a dose of 0.0606 Mrad/h

for periods varying between 1 and 8 hours (0.0606-0.4848 Mrad). The minimum concentrations necessary for the formation of gels at the irradiation times indicated above are reported in Fig. 1.

5 EXAMPLE 4:

1 gr of sodium alginate (medium viscosity) was dissolved in 20 ml of deionized, distilled water (5% w/v). To this solution were added 0.8 ml of glycidyl acrylate. The reaction was performed at ambient
10 temperature while stirring constantly. After 24 hours, the reaction was blocked by the addition of 4 ml of glycine at 20% w/v. The solution was then stirred for another 30 minutes, dialyzed for 48 hours in deionized, distilled water and lastly centrifuged at 3,000 rpm for
15 about 20 minutes. After dialysis, the final concentration of the solution proved to be about 1% w/v and the pH was 6. Solutions at concentrations of 1.5, 2, 2.5 and 3% were obtained by concentrating the solution to 1% in a rotor evaporator. Solutions at
20 different concentrations were acidified to pH=3 by the addition of citric acid, in such a way as to avoid any significant changes from the initial concentration of the polymer.

The purified solutions of functionalized polymer
25 were then gamma-irradiated at a dose of 0.0606 Mrad/h for periods varying between 1 and 8 hours (0.0606-0.4848 Mrad). The minimum concentrations necessary for the

formation of gels at the irradiation times indicated above are reported in Fig. 1.

The three-dimensional gels obtained from alginic acid were cut (1 cm^3) and dried at ambient temperature for 24 hours and at a temperature of 60°C for 12 hours. The samples, in threes, were weighed dry, left to swell in deionized, distilled water until their equilibrium had been reached, and then weighed again in their completely swollen state. The Q values relative to 1 and 8 hours of gamma-irradiation were 4.8-7.25 for 3% gels and 6.1-8.21 for 2.5% gels. The longer the gamma-irradiation time, the higher the Q value.

EXAMPLE 5:

1 gr of hyaluronic acid was dissolved in 20 ml of deionized, distilled water (5% w/v). To this solution was added 0.8 ml of glycidyl acrylate. The reaction was performed at ambient temperature while stirring constantly. After 24 hours, the reaction was blocked by the addition of 4 ml of glycine at 20% w/v. The solution was then stirred for another 30 minutes, dialyzed for 48 hours in deionized, distilled water and lastly centrifuged at 3,000 rpm for about 20 minutes. The final concentration of the solution proved to be about 1% w/v. Solutions at concentrations of 1.5, 2, 2.5 and 3% were obtained by concentrating the solution to 1% in a rotor evaporator.

The purified solutions of functionalized polymer were then gamma-irradiated at a dose of 0.0606 Mrad/h for periods varying between 1 and 8 hours (0.0606-0.4848 Mrad). The minimum concentrations necessary for the formation of gels at the irradiation times indicated above are reported in Fig. 2.

EXAMPLE 6:

1 gr of hyaluronic acid was dissolved in 20 ml of deionized, distilled water (5% w/v). To this solution was added 0.8 ml of glycidyl acrylate. The reaction was performed at ambient temperature while stirring constantly. After 24 hours, the reaction was blocked by the addition of 4 ml of glycine at 20% w/v. The solution was then stirred for another 30 minutes, dialyzed for 48 hours in deionized, distilled water and lastly centrifuged at 3,000 rpm for about 20 minutes. The final concentration of the solution proved to be about 1% w/v and the pH was 6. Solutions at concentrations of 1.5, 2, 2.5 and 3% were obtained by concentrating the solution to 1% in a rotor evaporator. The solutions at different concentrations were acidified to pH=3 by the addition of citric acid, in such a way as to avoid any significant changes from the initial concentration of the polymer.

The purified solutions of functionalized polymer were then gamma-irradiated at a dose of 0.0606 Mrad/h for periods varying between 1 and 8 hours (0.0606-0.4848

Mrad). The minimum concentrations necessary for the formation of gels at the irradiation times indicated above are reported in Fig. 2.

The three-dimensional gels obtained from hyaluronic acid were cut (1 cm^3) and dried at ambient temperature for 24 hours and at a temperature of 60°C for 12 hours. The samples, in threes, were weighed dry, left to swell in deionized, distilled water until their equilibrium had been reached, and then weighed again in their completely swollen state. The Q values relative to 1 and 8 hours of gamma-irradiation were 5.5-8.71 for 3% gels and 6.62-11.3 for 2% gels. The longer the gamma-irradiation time, the higher the Q value.

EXAMPLE 7:

1 gr of the benzyl ester of hyaluronic acid, partially esterified (25%), was dissolved in 20 ml of deionized, distilled water (5% w/v). To this solution were added 0.8 ml of glycidyl acrylate. The reaction was performed at ambient temperature while stirring constantly. After 24 hours, the reaction was blocked by the addition of 4 ml of glycine at 20% w/v. The solution was then stirred for another 30 minutes, dialyzed for 48 hours in deionized, distilled water and lastly centrifuged at 3,000 rpm for about 20 minutes. The final concentration of the solution proved to be about 1% w/v and the pH was 6. Solutions at concentrations of 1.5, 2, 2.5 and 3% were obtained by

concentrating the solutions to 1% in a rotor evaporator.

The purified solutions of functionalized polymer were then gamma-irradiated at a dose of 0.0606 Mrad/h for periods varying between 1 and 8 hours (0.0606-0.4848 Mrad). The minimum concentrations necessary for the formation of gels at the irradiation times indicated above are reported in Fig. 3.

EXAMPLE 8:

1 gr of the benzyl ester of hyaluronic acid, partially esterified (25%), was dissolved in 20 ml of deionized distilled water (5% w/v). To this solution was added 0.8 ml of glycidyl acrylate. The reaction was performed at ambient temperature while stirring constantly. After 24 hours, the reaction was blocked by the addition of 4 ml of glycine at 20% w/v. The solution was then stirred for another 30 minutes, dialyzed for 48 hours in deionized distilled water and lastly centrifuged at 3,000 rpm for about 20 minutes. The final concentration of the solution proved to be about 1% w/v and the pH was 6. Solutions at concentrations of 1.5, 2, 2.5 and 3% were obtained by concentrating the solution to 1% in a rotor evaporator. The solutions at different concentrations were acidified to pH=3 by the addition of citric acid, in such a way as to avoid any significant changes from the initial concentration of the polymer.

The purified solutions of functionalized polymer were then gamma-irradiated at a dose of 0.0606 Mrad/h for periods varying between 1 and 8 hours (0.0606-0.4848 Mrad). The minimum concentrations necessary for the formation of gels at the irradiation times indicated above are reported in Fig. 3.

The three-dimensional gels obtained from HA-25 were cut (1 cm^3) and dried at ambient temperature for 24 hours and at a temperature of 60°C for 12 hours. The samples, in threes, were weighed dry, left to swell in deionized, distilled water until their equilibrium had been reached, and then weighed again in their completely swollen state. The Q values relative to 1 and 8 hours of gamma-irradiation were 4-5.62 for 2.5% gels and 4.6-5.95 for 2% gels. The longer the gamma-irradiation time, the higher the Q value.

The invention being thus described, it is clear that these methods can be modified in various ways. Said modifications are not to be considered as divergences from the spirit and purpose of the invention and any modification which would be apparent to an expert in the field comes within the scope of the following claims.

CLAIMS

- 1 1. Process for the preparation of biocompatible
2 hydrogels which includes the following steps:
 - 3 (a) reacting a polyelectrolyte polysaccharide with
4 a functionalizing agent in order to introduce
5 double bonds into the structure of the
6 polysaccharide and produce a functionalized
7 polysaccharide;
 - 8 (b) acidifying the functionalized polysaccharide
9 of step (a); and
 - 10 (c) subjecting the acidified polysaccharide of
11 step (b) to gamma-irradiation in order to form
12 crosslinked bonds so as to produce the
13 biocompatible hydrogel.

- 1 2. Process for the preparation of hydrogels
2 according to claim 1, wherein the functionalizing agent
3 is glycidyl acrylate which is present in an amount of
4 between 0.8 and 1.3 ml/1 g of the polyelectrolyte
5 polysaccharide.

1 3. Process for the preparation of hydrogels
2 according to claims 1 to 2, wherein the functionalized
3 polysaccharide in step (b) is purified by dialysis.

1 4. Process for the preparation of hydrogels
2 according to claims 1 to 3, wherein the gamma-
3 irradiation is provided at a dose of 0.0606 to 0.485
4 Mrad.

1 5. Process for the preparation of hydrogels
2 according to claims 1 to 4, wherein the functionalized
3 polysaccharide is acidified in step (b) at a pH of
4 between 3 and 6.

1 6. Process for the preparation of hydrogels
2 according to claim 1, wherein the polyelectrolyte
3 polysaccharide is alginic acid.

1 7. Process for the preparation of hydrogels
2 according to claim 1, wherein the polyelectrolyte
3 polysaccharide is hyaluronic acid.

1 8. Process for the preparation of hydrogels
2 according to claim 1, wherein the polyelectrolyte
3 polysaccharides are partially esterified benzyl esters
4 of hyaluronic acid.

1 9. Biocompatible hydrogels produced according to
2 claims 1 to 8 for the preparation of biomaterials for
3 medical-surgical, pharmaceutical and cosmetic uses.

1 10. Three-dimensional biocompatible hydrogels
2 produced according to claims 1 to 9 for the preparation
3 of membranes, fibers, films, threads and gauzes.

1 11. Three-dimensional biocompatible hydrogels
2 produced according to claims 1 to 10 for the preparation
3 of controlled release systems for active principles such
4 as proteins, growth factors, enzymes, drugs and
5 biologically active substances for topical and oral
6 uses.

1 12. Biocompatible hydrogels in the form of visco-
2 elastic solutions according to claims 1 to 9 for the
3 preparation of controlled release systems for active
4 principles such as proteins, growth factors, enzymes,
5 drugs and biologically active substances for topical,
6 sub-cutaneous, intramuscular and intravenous uses.

1 13. Biocompatible hydrogel which comprises
2 functionalized polyelectrolyte polysaccharide molecules
3 crosslinked by exposure to gamma-irradiation.

1 14. Biocompatible hydrogel according to claim 13,
2 which further comprises an active principle such as
3 proteins, growth factors, enzymes, drugs and
4 biologically active substances for topical, sub-
5 cutaneous, intramuscular and intravenous uses.

1 15. Biocompatible hydrogel according to claims 13
2 to 14, wherein the swelling ratio Q value is from 4 to
3 11.3.

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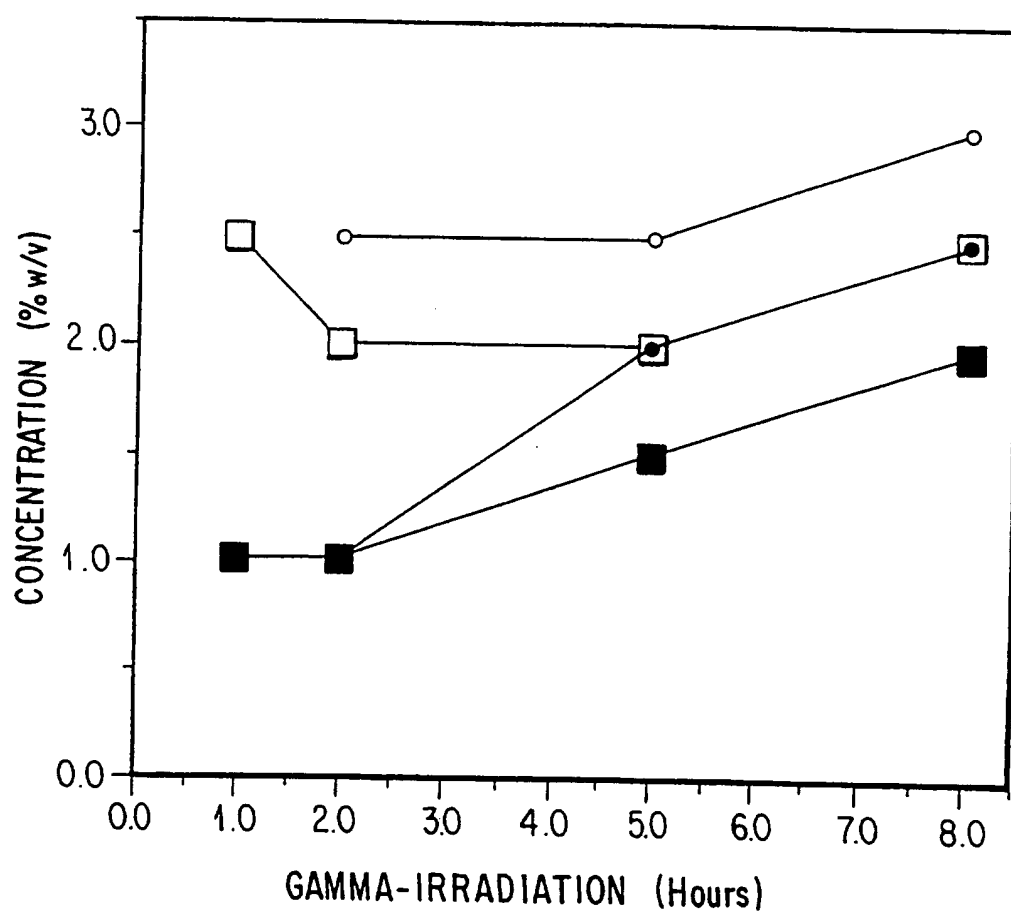


FIG.1

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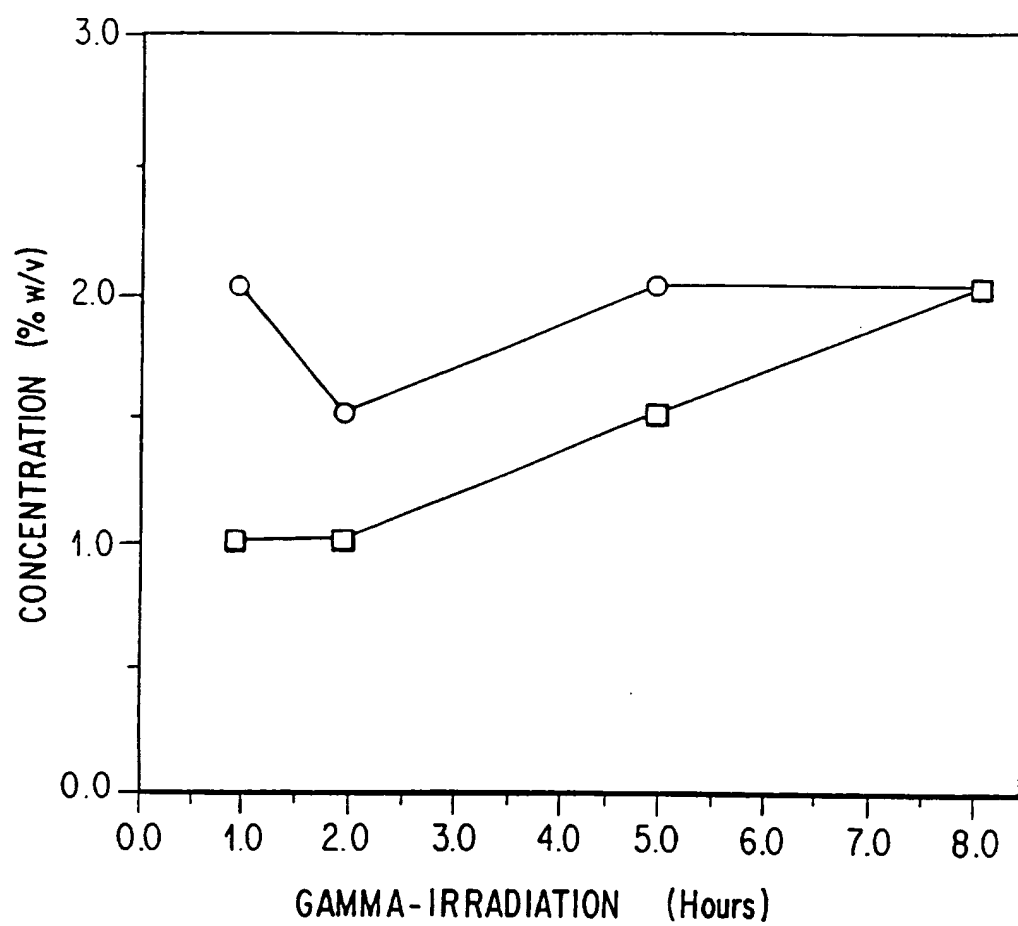


FIG.2

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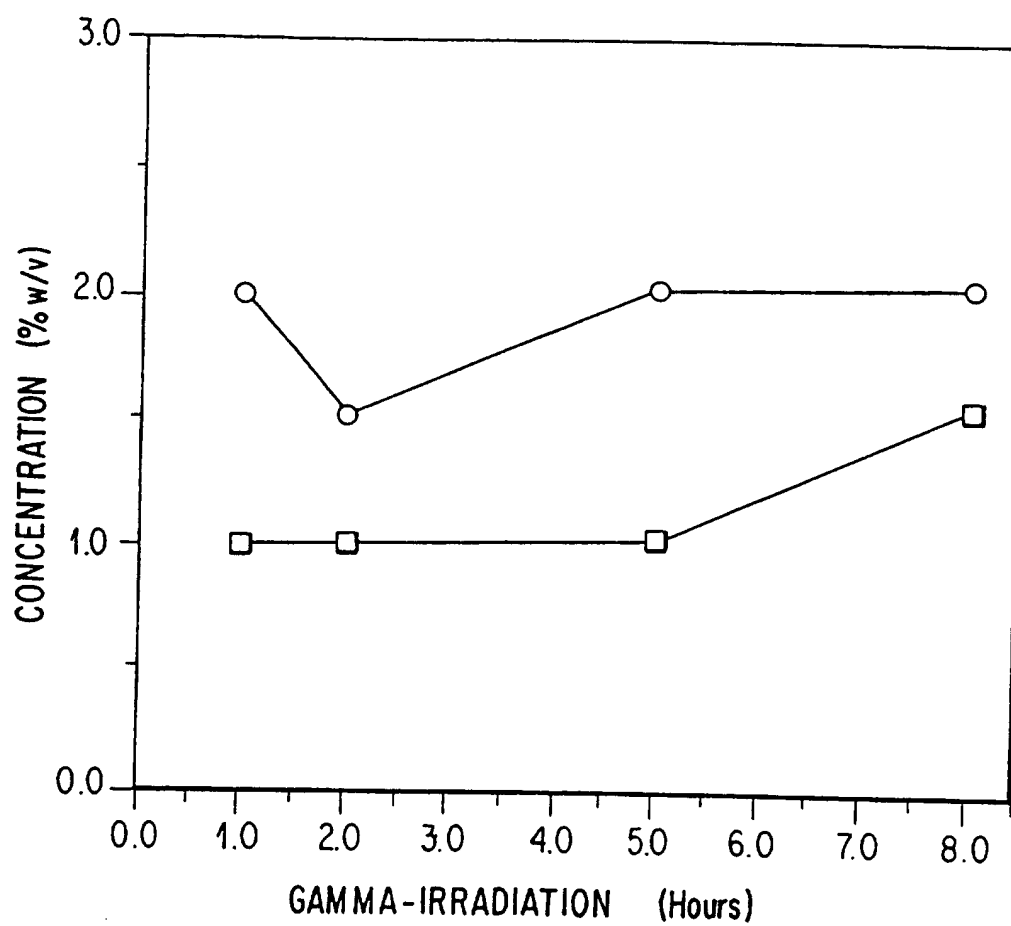


FIG. 3

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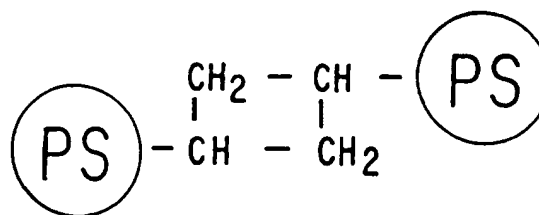
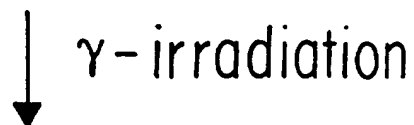
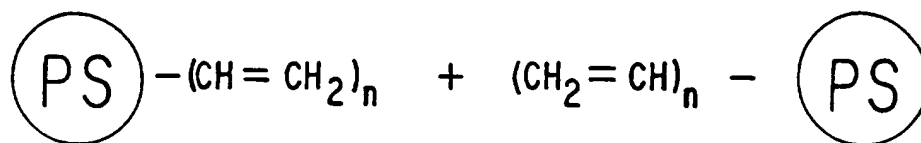
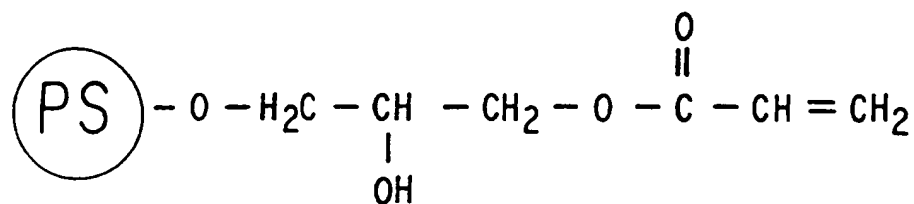
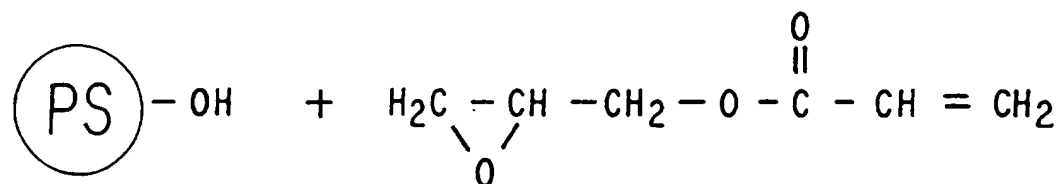


FIG. 4

A. CLASSIFICATION OF SUBJECT MATTER A 61 K 41/00, A 61 K 47/32, A 61 K 47/36, A 61 K 9/02, A 61 K 9/06, A 61 K 9/22, C 08 F 2/46, //A 61 K 38/00 According to International Patent Classification (IPC) or to both national classification and IPC 6		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) A 61 K, C 08 F, C 08 B Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	K.R. KAMATH et al. "Preparation and Character- ization of Enzyme-Digestible Hydrogels from Natural Poly- mers by Gamma Irradiation", 204th National Meeting of the ACS, ACS Symposium 1992, cited in ACS Symposium Series 545, American Chemical Society,	1, 2, 4, 9, 11-15
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 26 September 1995		Date of mailing of the international search report 13.10.95
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patendaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016		Authorized officer MAZZUCCO e.h.

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	<p>pages 198-199, especially page 198, left column, line 16 - page 199, left column; 8th line after fig. 41.</p> <p>-----</p>	

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to the International Search
Report to the International Patent
Application No.

ANNEX

ANNEXE

au rapport de recherche inter-
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